

## Assessment of upper airway stabilizing forces with the use of phrenic nerve stimulation in conscious humans

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**Sériès, Frédéric, and Germain Éthier.** Assessment of upper airway stabilizing forces with the use of phrenic nerve stimulation in conscious humans. *J Appl Physiol* 94: 2289–2295, 2003. First published February 21, 2003; 10.1152/japplphysiol.00924.2002.—Phrenic nerve stimulation (PNS) applied at end-expiration allows the investigation of passive upper airway (UA) dynamic during wakefulness. Assuming that phasic UA dilating/stabilizing forces should modify the UA properties when twitches are applied during inspiration, we compared the UA dynamic responses to expiratory and inspiratory twitches (2 s and 200 ms after expiratory and inspiratory onset, respectively) in nine men (mean age 28 yr). This procedure was repeated with a 2-cm mouth opening provided with a closed mouthpiece. The percentage of flow-limited (FL) twitches was significantly higher when PNS was realized during expiration than during inspiration. Maximal inspiratory flow ( $\dot{V}_{I_{max}}$ ) of FL twitches was significantly higher for inspiratory twitches ( $1,383 \pm 42$  and  $1,185 \pm 40$  ml/s). With mouth aperture,  $\dot{V}_{I_{max}}$  decreased with an increase in the corresponding pharyngeal resistance values, and the percentage of twitch with a FL regimen increased but only for inspiratory twitches. We conclude that 1) UA dynamics are significantly influenced by the inspiratory/expiratory timing at which PNS is applied, 2) the improvement in UA dynamic properties observed from expiratory to inspiratory PNS characterizes the overall inspiratory stabilizing effects, and 3) mouth aperture alters the stability of UA structures during inspiration.

upper airway dilator muscles; airway closure; upper airway stability

AMONG THE DIFFERENT TASKS of the upper airway (UA) structures, several are dedicated to respiratory functions. UA contributes to determine the resistance of the respiratory system and the respiratory workload (8). The laryngeal component of the UA structure is also involved in the regulation of end-expiratory lung volume (1). During sleep, the maintenance of UA patency is critical to prevent the occurrence of obstructive breathing disorders that lead to sleep, hemodynamic, and autonomic nervous system disturbances. The ability of UA to adequately achieve these different purposes depends on the characteristics of UA tissue structure (e.g., UA shape and dimension, impact of gravity on these tissues) (13) and function (e.g., efficiency of phasic UA inspiratory forces). Thus the net

mechanical effects of UA dilator muscles' tonic and phasic activities depend on the features of structural and functional components (5, 20). Electromyogram (EMG) recordings are used to assess the dilating function of UA muscles. However, EMG recordings obviously do not take into account the effective UA dilating force generated in vivo because it depends on UA muscle contractile properties and on the characteristics of the surrounding soft tissues on which these dilating/stabilizing forces are applied. Furthermore, the overall dilating effect of UA muscle contraction results from the synergistic interaction between its respective mechanical function that cannot be assessed by the recording of isolated muscle activity. There are, therefore, presently no ways to evaluate the efficiency of UA dilating/stabilizing forces.

We have recently described the advantages of using the phrenic nerve stimulation (PNS) method to evaluate UA dynamic properties of nonphasically active UA muscles during wakefulness (26). Application of the twitch stimulus at end-expiration (when there is no phasic activity of UA dilator muscles) in subjects quietly breathing by the nose is able to induce a clear flow limitation (FL) pattern, where the collapsing site is located at the pharyngeal level (27). During inspiration, the activation of UA muscles is aimed at increasing UA stiffness and stability. Therefore, UA dynamics would be expected to differ when PNS is applied during inspiration compared with expiration. Gottfried et al. (6) previously found that the PNS-induced rise in UA resistance is dramatically influenced by the respiratory timing at which it is applied, with an ~100% increase in peak flow resistance when stimulation is applied during expiration as opposed to inspiration. However, because of the type of PNS (tetanic electric stimulation) and the study conditions (animals anesthetized with hyperextension of the neck and the tongue secured), these results cannot be directly used to document the efficiency of dilating/stabilizing forces in awake humans.

Therefore, by postulating that the difference in UA mechanical properties evaluated with PNS between these two conditions would reflect the net effect of UA dilating/stabilizing forces applied during inspiration,

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the aims of the present study were to investigate to what extent the respiratory timing (inspiration vs. expiration) at which PNS is applied influences the pressure-flow relationship in awake normal subjects. We tested the ability of the investigated technique to evaluate the effects of a functional disturbance of UA dilator function represented by mouth opening. This was done because we have previously demonstrated that mouth aperture increases UA instability during sleep (16) and that this could be due to a decrease in the mechanical efficiency of UA muscle contraction.

## MATERIALS AND METHODS

**Subjects.** Nine men (age:  $28 \pm 8$  yr; body mass index:  $23.2 \pm 2.3$  kg/cm<sup>2</sup>; neck circumference:  $37.4 \pm 2.1$  cm; means  $\pm$  SE) participated in the study. They were not taking any medication, did not snore, and did not complain of any symptoms suggestive of sleep-related breathing disorders. The internal review board of our institution approved this protocol, and informed consent was obtained from each subject.

**Characterization of UA dynamics.** Surface recordings of the right and left costal diaphragmatic EMG activities were obtained by silver cup electrodes placed on the axillary line of the sixth to the eighth right and left intercostal spaces and connected to a electromyograph (Biopac, Santa Barbara, CA). A pressure-tipped catheter (model CT/S X1058, Gaeltec, Hackensack, NJ) was inserted through one nare after local anesthesia (1 ml of 2% viscous xylocaine) and located at 16 cm from the nares to record hypopharyngeal (retroglossal) pressure (Pphar) (8). A plastic nasal stent (Nozovent; WPM International AB; Göteborg, Sweden) was placed in the anterior nares to prevent nasal collapse, and the catheter was secured on the nose. A tight-fitting nasal continuous positive-pressure mask (Profile<sup>®</sup> Light Nasal Mask, Respirationics, Pittsburgh, PA) was then placed over the nose. Occlusion of its opening during maximal inspiratory efforts assessed its airtightness. A second catheter was passed through another opening of the mask to measure pressure inside the mask. Flow was obtained from a pneumotachograph (model 112467-3850A, Hans Rudolph, Kansas City, MO) connected to the mask. Pressures and flow were digitally recorded at a 300-Hz sample rate (Digidata 1320, Axon Instrument, Foster City, CA). The patients were studied seated in a comfortable armchair with a 60° inclination and with their head maintained in a natural, "neutral" position by a moulded pillow to ascertain that head and neck position remained unchanged during the experiment.

**PNS.** Bilateral anterior magnetic PNS (BAMPS) was performed with two Magstim 200 stimulators (Magstim, Whitland, Dyfed, UK), connected to two 90° handle, 45-mm figure eight-shaped coils, according to previously described technique (18, 30). In brief, each stimulating coil was positioned anterolaterally over the anatomical landmark of the phrenic nerve in the neck and at the posterior border of the sternomastoid muscle at the level of the cricoid cartilage, with the handle of the coil making a 45° axis with both the midsagittal plane of the body and the horizontal plane. The intensity of stimulation was set at the maximal possible output of the stimulators. A simplified recruitment curve (motor response to stimulation against stimulation intensity) was performed to verify the supramaximal nature of the stimulation. The two stimulators were triggered by a timer driven by the changes in flow direction. The twitches were delivered after

the operator-selected delay after inspiration or expiration onset had been reached.

**Study design.** All measurements were made with the subjects breathing exclusively by the nose. The optimal position and orientation of the coils were determined separately for each side at 80–100% stimulation intensity. BAMPS was applied at end-expiration (2 s after expiratory onset) or early inspiration (200 ms after inspiratory onset) in random order. Subjects were blind to the twitch timing. For each respiratory timing, one series of five stimulations was applied with a 5% stepwise increase in intensity from 65 to 100% of maximal stimulation intensity. Once this initial procedure had been completed, the protocol was repeated with and without an occluded mouth piece (20-mm distance between incisors). For convenience, the stimulation intensity levels tested for this part of the study were limited to 70, 85, and 100% maximum intensity. The recording sessions with and without mouthpiece were realized in random order. Special attention was paid to optimally cover the mouthpiece by the cheeks and lips to ascertain airtightness. This was assessed by verifying that the subjects could not breathe by the mouth with the mouthpiece in place.

**Data and statistical analyses.** The twitch-induced breaths were considered flow limited when instantaneous flow plateaued or decreased despite a persistent increase in driving pressure. Representative tracings of the Pphar and flow responses to twitch are represented in Fig. 1. The following variables were measured: 1) maximal inspiratory flow ( $\dot{V}_{I_{max}}$ ), 2) maximal instantaneous flow of flow-limited twitches ( $\dot{V}_{I_{max,lim}}$ ), 3) Pphar at 400 ml/s, 4) Pphar at  $\dot{V}_{I_{max}}$  (Pphar limit for twitches associated with FL), 5) peak Pphar ( $Pphar_{max}$ ), 6) minimal flow with increasing driving pressure during flow-limited twitches ( $\dot{V}_{I_{min}}$ ), 7) the drop in inspiratory flow from  $\dot{V}_{I_{max,lim}}$  to  $\dot{V}_{I_{min}}$  at  $Pphar_{max}$  for twitches associated with FL ( $\Delta\dot{V}_I$ ), 8) the corresponding pharyngeal resistance value, and 9) the percentage of twitches associated with flow limitation.  $\dot{V}_{I_{max,lim}}$ ,  $\dot{V}_{I_{min}}$ , Pphar of flow-limited twitches ( $Pphar_{lim}$ ), and  $\Delta\dot{V}_I$  were considered to characterize FL twitches.

For data analysis, two statistical approaches were performed. First, the split-plot design was completed to separately analyze the effects of stimulation intensity and twitch respiratory timing on the measured variables. Because stimulation intensity increased repeatedly for each subject, the respiratory timing factors were randomly assigned to the subjects (main plot) and stimulation intensity factors were assigned to split plot. This second factor was analyzed as a repeated-measures factor. A mixed model analysis was performed with an interaction term between the stimulation intensity and respiratory timing factors. A first-order autoregressive covariance structure was used to evaluate the influence of stimulation intensity and timing on the measured variables (4). The univariate normality assumptions were verified with Shapiro-Wilk tests, and multivariate normality was verified with Mardia tests (15). To evaluate the effects of the mouthpiece, a new fixed factor was introduced into the first model. Interaction terms were included in the statistical model, and a similar approach was used to analyze variables. The same covariance structure was used because the three stimulation levels were equally represented. The results of these statistical procedures take into account for the multiple nature of the comparisons. The results were considered significant with *P* values of  $\leq 0.05$ . All analyses were conducted by using the statistical package SAS (SAS Institute, Cary, NC).

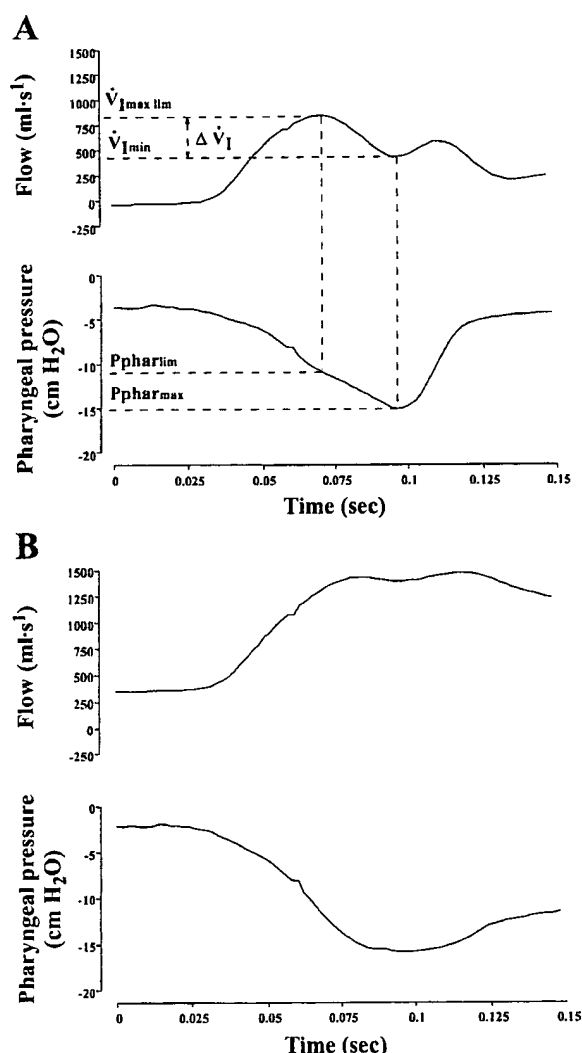


Fig. 1. Representative examples of the pharyngeal pressure (Pphar) and flow responses to twitch applied during expiration (A) and inspiration (B) in 1 subject. The pressure-flow pattern obtained when the twitch is applied during expiration is characteristic of a flow-limited regimen. Positive flow values represent inspiratory flow.  $\dot{V}_{I_{max,lim}}$ , maximal inspiratory flow of flow-limited twitches;  $\dot{V}_{I_{min}}$ , minimal inspiratory flow;  $P_{phar,lim}$ , Pphar of flow-limited twitches;  $P_{phar,max}$ , maximal Pphar.

## RESULTS

$\dot{V}_{I_{max}}$  and  $P_{phar,max}$  were significantly higher for inspiratory than expiratory twitches (Table 1). The different variables did not change with stimulation intensity. No difference was observed in pharyngeal resistance measured at  $\dot{V}_{I_{max}}$ , at 400 ml/s and at  $P_{phar,max}$  (Table 1) between the two-twitch respiratory timing. No FL breaths were seen during spontaneous breathing. The percentage of FL twitches was significantly higher when BAMPS was delivered during expiration than during inspiration (Table 1). The pattern of flow limitation significantly differed between the twitches applied at the two respiratory times. Twitch applied at end-expiration was accompanied by a clear drop in flow, whereas Pphar continued to decrease down to  $P_{phar,max}$  (Figs. 1 and 2). For twitches applied during inspiration,  $\dot{V}_{I_{max,lim}}$  was higher (Figs. 1 and 2) and the flow plateauing was reached later, resulting in a short plateau and a limited drop in flow. The difference in  $\dot{V}_{I_{max,lim}}$  between expiratory and inspiratory twitches was not influenced by stimulation intensity (Fig. 3; Table 1). No difference in the change in inspiratory flow was found between the two-twitch timing (Table 1). The Pphar at which a dissociation between flow and Pphar was observed ( $P_{phar,lim}$ ) was reached later during the twitch applied during inspiration than during end-expiration (Figs. 1 and 2). As a consequence,  $P_{phar,lim}$  was significantly more negative during inspiratory than during expiratory twitches at all the stimulation intensities (Fig. 4; Table 1).

Although maintaining an exclusive nasal-breathing route, mouth opening significantly altered UA dynamic characteristics. For both inspiratory and expiratory twitches, the closed mouthpiece decreased  $\dot{V}_{I_{max}}$  and increased the corresponding pharyngeal resistance values (Table 2). This difference was observed at the three tested stimulation intensity levels. There was a tendency for  $\dot{V}_{I_{max,lim}}$  to decrease with the mouthpiece in place, but the difference was borderline significant ( $P = 0.07$ ). This effect was not different for inspiratory and expiratory twitches. No difference in pharyngeal resistance at 400 ml/s or in peak pressure resistance was observed.  $P_{phar,lim}$  and  $P_{phar,max}$  values were not significantly different without and with the mouth-

Table 1. Values of BAMPS-induced flow and pressure characteristics for expiratory and inspiratory twitches

	Inspiration	Expiration	P Value for Interaction with BAMPS Timing	P Value for Interaction with Stimulation Intensity
$\dot{V}_{I_{max}}$ , ml/s	1,461 ± 30	1,182 ± 33	0.002	0.09
$P_{phar,max}$ , cmH <sub>2</sub> O	-12.9 ± 0.45	-9.6 ± 0.48	0.005	0.8
R at 400 ml/s, cmH <sub>2</sub> O·l <sup>-1</sup> ·s	6.2 ± 0.5	6.1 ± 0.5	0.2	0.5
R $\dot{V}_{I_{max}}$ , cmH <sub>2</sub> O·l <sup>-1</sup> ·s	6.2 ± 0.3	5.6 ± 0.4	0.3	0.2
R $P_{phar,max}$ , cmH <sub>2</sub> O	11.8 ± 1.1	14.6 ± 1.4	0.9	0.8
$\dot{V}_{I_{max,lim}}$ , ml/s	1,383 ± 42	1,185 ± 40	0.04	0.4
$\Delta \dot{V}_I$ , ml/s <sup>-1</sup>	138 ± 36	217 ± 24	0.2	0.1
$P_{phar,lim}$ , cmH <sub>2</sub> O	-10.5 ± 0.5	-8.5 ± 0.4	0.04	0.4
%Twitches with FL	45.0 ± 4.3	60.9 ± 4.3	0.03	0.8

Values are means ± SE.  $\dot{V}_{I_{max}}$ , maximal inspiratory flow;  $P_{phar,max}$ , peak hypopharyngeal pressure; R, resistance;  $\dot{V}_{I_{max,lim}}$ , maximal instantaneous flow of flow-limited twitches;  $\Delta \dot{V}_I$ , drop in isometric flow from  $\dot{V}_{I_{max}}$  to  $P_{phar,max}$ ; FL, flow limitation. BAMPS, bilateral anterior magnetic phrenic nerve stimulation.

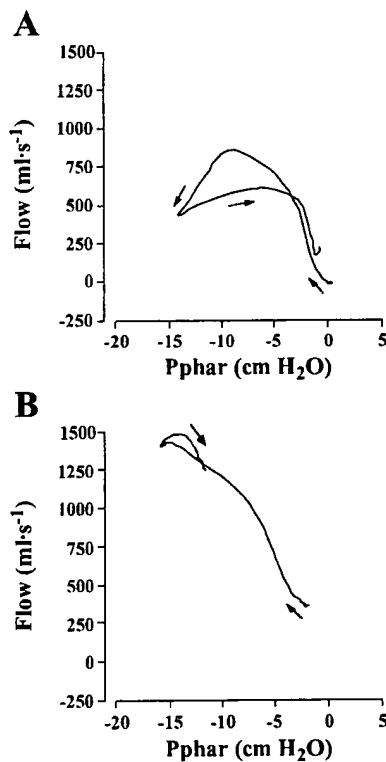


Fig. 2. Flow-pressure curves obtained from tracings represented in Fig. 1. When the twitch is applied at end-expiration (A), a clear drop in flow occurs with increasing pharyngeal pressure once  $\dot{V}_{I_{max,lim}}$  has been reached. When the twitch is applied during inspiration (B), only a short plateauing and a small decrease in flow are observed.

piece (Table 2). The mouth piece increased the percentage of twitch with a FL regimen only for inspiratory twitches; no difference in this parameter was found when inspiratory and expiratory results were pooled (Table 2). Furthermore, this effect was predominantly observed at maximal stimulation intensity. At 70 and 80% stimulation intensity, the percentage of twitches with FL was higher during expiratory than during

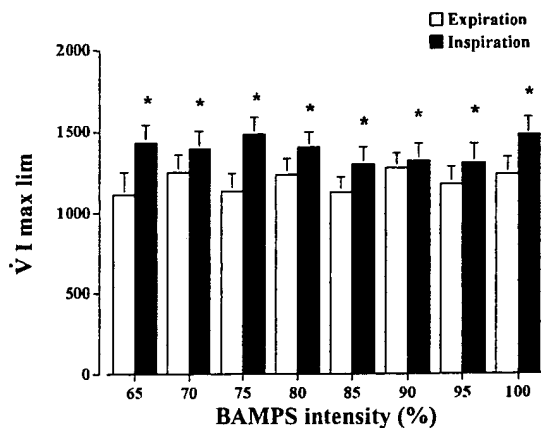


Fig. 3. Mean  $\pm$  SE values of  $\dot{V}_{I_{max,lim}}$  for bilateral anterior magnetic phrenic nerve stimulation (BAMPS) applied at end-expiration and early inspiration for each stimulation intensity. The maximal flow value that was reached was always higher when BAMPS was applied during inspiration. \*Inspiratory and expiratory values that are significantly different ( $P < 0.05$ ).

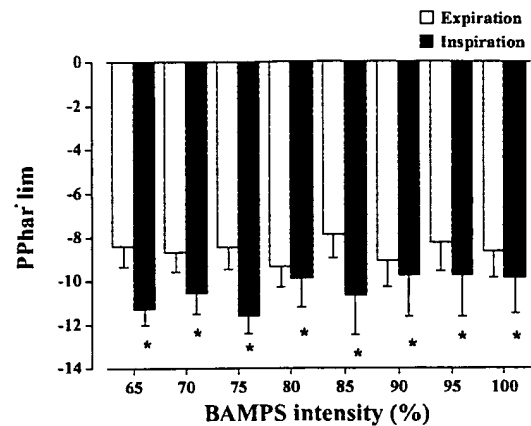


Fig. 4. Mean  $\pm$  SE values of  $P_{phar_{lim}}$  for BAMPS applied at end-expiration and early inspiration for each stimulation intensity. The pressure at which flow limitation occurred was always more negative when BAMPS was applied during inspiration. \*Inspiratory and expiratory values that are significantly different ( $P < 0.05$ ).

inspiratory stimulation both without and with the mouthpiece (Fig. 5). This pattern was dramatically modified at 100% stimulation intensity, where the percentage of FL twitches became higher during inspiration than during expiration (Fig. 5).

## DISCUSSION

Our results demonstrate that the timing at which the stimulus is applied significantly influences the UA dynamic characteristics assessed by PNS. Until now, we have used the PNS model during expiration to explore the properties of UA free of phasic activity and thus when there are no inspiratory dilating/stabilizing forces applied to the UA. This PNS timing allows the investigation of UA mechanical properties whose stability only relies on UA muscle tonic activity.

We have previously used this model to investigate UA collapsibility while awake (21) to quantify the effects of changing tonic muscle activity (29) as well as neck position (40) and to quantify the influence of CPAP on the UA dynamics features (30). Use of the PNS procedure during inspiration compared with data obtained during expiration adds an entirely novel evaluation of UA dynamics since it investigates the overall effect of UA dilator contraction on UA stability. This information is not provided by measurements of the tonic and phasic EMG activities of UA dilator muscles (7, 17) because it may be difficult to extrapolate UA EMG recordings to their dilating function [i.e., in the case of eccentric contraction where muscle lengthening occurs during phasic EMG activation (2)]. A second important aspect relates to the influence of the mechanical conditions that prevail at the time of the UA muscle contraction on its mechanical efficiency [i.e., lung volume (12), UA hysteresis (23), synergistic effect of agonistic/antagonists muscles (11)]. This last aspect is illustrated by the enhancement of the mechanical effect of dilator contraction when activation is combined compared with when either muscle contracts alone (9, 34) and when it occurs concurrently with

Table 2. BAMPS-induced flow and pressure characteristics for expiratory and inspiratory twitches without and with mouthpiece

	Inspiratory		Expiratory		P Value for Interaction with MP	P Value for Interaction with BAMPS Timing and with MP
	Without MP	With MP	Without MP	With MP		
$\dot{V}_{I\max}$ , ml/s	1,441 $\pm$ 48	1,355 $\pm$ 60	1,182 $\pm$ 47	1,087 $\pm$ 58	0.05	0.8
$P_{phar, \max}$ , cmH <sub>2</sub> O	-12.9 $\pm$ 0.7	-14.7 $\pm$ 0.8	-9.8 $\pm$ 0.8	-10.1 $\pm$ 0.5	0.1	0.08
R 400 at ml/s, cmH <sub>2</sub> O $\cdot$ l <sup>-1</sup> $\cdot$ s	5.4 $\pm$ 0.7	8.1 $\pm$ 0.6	6.6 $\pm$ 0.5	6.8 $\pm$ 0.6	0.2	0.2
R $\dot{V}_{I\max}$ , cmH <sub>2</sub> O $\cdot$ l <sup>-1</sup> $\cdot$ s	6.0 $\pm$ 0.5	8.0 $\pm$ 0.6	5.4 $\pm$ 0.5	7.0 $\pm$ 0.7	0.006	0.5
R $P_{phar, \max}$ , cmH <sub>2</sub> O $\cdot$ l <sup>-1</sup> $\cdot$ s	11.5 $\pm$ 1.4	12.3 $\pm$ 2.9	14.3 $\pm$ 2.9	10.6 $\pm$ 1.7	0.3	0.6
$\dot{V}_{I\max, \lim}$ , ml/s	1,350 $\pm$ 62	1,292 $\pm$ 62	1,177 $\pm$ 59	1,021 $\pm$ 74	0.07	0.4
$\Delta \dot{V}_I$ , ml/s	108 $\pm$ 48	129 $\pm$ 51	209 $\pm$ 39	142 $\pm$ 26	0.08	0.08
$P_{phar, \lim}$ , cmH <sub>2</sub> O	-10.5 $\pm$ 0.9	-11.9 $\pm$ 0.5	-8.5 $\pm$ 0.6	-8.4 $\pm$ 0.6	0.2	0.2
%Twitches with FL	44.4 $\pm$ 7.1	49.0 $\pm$ 7.5	61.8 $\pm$ 6	62.1 $\pm$ 7.1	0.8	0.04

Values are means  $\pm$  SE. MP, mouthpiece.

tracheal traction (36, 28, 35). From a physiological point of view, the dilating force transmitted to UA tissues largely depends on the physiological properties of UA dilator muscles (24) and on the compliance of the surrounding soft tissues (25). Taken together, the above-mentioned factors make it very difficult to assess the effective stabilizing effect of phasic activation of UA dilators. Until the present, there were no methods available to measure this effect. Application of the PNS technique during inspiration to the model developed to explore nonphasically active UA provides a unique condition to explore the effects of all the forces that interact with UA stability during inspiration; the only difference between inspiratory and expiratory twitches is the inspiratory UA traction forces. Furthermore, PNS takes into account the efficiency of muscle contraction to generate tension, the transmission of this tension to surrounding tissues, and the effects of the inspiratory tracheal traction.

From a methodological point of view, our results could be criticized because of the absence of EMG recording and the respiratory timing parameters at which BAMPs was realized. Although not measured in

the present study, the preinspiratory activation of UA muscles has been well described in a large number of dilator muscles, such as alae nasi (33), soft palate muscles (levator veli palatini, tensor veli palatini, palatoglossus, musculus uvulae) (37), genioglossus (19), and posterior cricoarythenoid (39), with preactivation intervals varying from 92 to 220 ms. Furthermore, the activation pattern differs between respiratory and UA dilator muscles, with the rate of rise being higher in the latter muscles, which rapidly reach a plateau, whereas the former muscles progressively increase throughout inspiration (19, 37, 39). Because of this, most of the inspiratory shortening of these dilator muscles should have occurred at the time inspiratory BAMPs was applied (3, 38). Also, no genioglossus phasic activity is recorded when PNS is applied at end-expiration (27). There is, therefore, a body of evidence that the UA dilator muscle activation pattern strongly differed between two BAMPs timings and that UA muscle recruitment was maximal (or almost) during inspiratory BAMPs. The question can be raised about the influence of the negative pressure-induced rise in UA muscle activity on the different UA dynamic patterns that we observed. Our laboratory (26) previously reported that such an increase in genioglossus activity occurs when the peak negative pressure and the largest drop in flow have been reached. It is very improbable that this reflex-mediated activation of UA dilators influenced the  $\dot{V}_{I\max, \lim}$  and limited-twitch  $P_{phar}$  values that are reached earlier after the twitch. Therefore, even if this reflex response can be expected to be larger during inspiratory than expiratory twitches (especially in the context of an early inspiratory twitch) (41), this should not account for the difference in  $\dot{V}_{I\max, \lim}$  and limited-twitch  $P_{phar}$  between these two conditions because of the delay in this reflex response.

The inspiratory timing that was chosen was also justified by the need to perform inspiratory and expiratory BAMPs at similar lung volume values. This is a critical issue because lung volume significantly interacts with UA mechanical properties. When expiratory twitches are performed 2 s after expiratory onset and inspiratory twitches after a 200-ms inspiratory delay, it kept the differences in lung volume minimal but

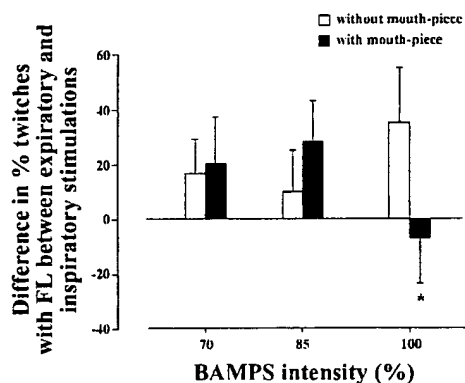


Fig. 5. Difference in the percentage of twitches presenting a flow-limitation (FL) regimen between BAMPs performed during expiration and inspiration. At 70 and 80% stimulation intensity, the percentage of twitches with FL was higher during expiratory than inspiratory stimulation both without and with a mouthpiece. At 100% stimulation intensity, the percentage of FL twitches became higher during inspiration than during expiration. \*Inspiratory and expiratory values that are significantly different ( $P < 0.05$ ).

allowed the evaluation of the tracheal traction effect in a quasi-isometric situation.

When the difference in the studied parameters observed between inspiratory and expiratory twitches is explained, two different factors should be considered: 1) the effects of respiratory timing on the efficacy of diaphragmatic stimulation and 2) the changes in UA stability from one condition to the other. The difference in peak pharyngeal pressure observed between the inspiratory and expiratory PNS timing could relate to the improvement in the ability to generate negative downstream pressure when the twitch is applied once inspiratory muscles are activated as a consequence of the mechanical conditions that prevail during inspiratory twitches. The transdiaphragmatic pressure generated with PNS is less with transcutaneous electric stimulation than with cervical magnetic stimulation tested in static (14) or dynamic conditions (30). Such a difference is attributed to the activation of postural and cervical accessory muscles (31) that stabilize the rib cage and improve the effectiveness of diaphragmatic contraction. However, there is no activation of the abdominal muscles or of lower intercostals with these PNS techniques (14). Because these muscle groups were also activated at the time BAMPS was applied during inspiration, it is reasonable to conceive that the rib cage stabilization effect was further enhanced, thereby allowing the development of more negative twitch pressures. It is noteworthy that this effect goes beyond the potential detrimental effect of the increase in lung volume on twitch-induced transdiaphragmatic pressure (32), which further supports the minimal effects of the differences in lung volume between expiratory to inspiratory twitches.

Noteworthy is the absence of significant improvement in pharyngeal resistance between inspiratory and expiratory twitches. However, it is important to emphasize that this absence of change in resistance occurred in parallel with an increase in the pharyngeal pressure measured at  $\dot{V}_{I_{\max}}$  and  $\dot{V}_{I_{\min}}$ . Because the flow-limited regimen is associated with the largest pressure drop for a given change in flow, it is remarkable to observe an absence of change in resistance between the two twitch times when the reached pressure levels increase by 30% during inspiratory twitch (Table 1). Furthermore, the threefold increase in pharyngeal resistance from  $\dot{V}_{I_{\max}}$  to  $\dot{V}_{I_{\min}}$  during expiratory twitches went to a twofold increase in these resistances during inspiratory twitches (Table 1). Thus the absence of rise in resistance in the context of an increase in the corresponding pressures with inspiratory twitches may be interpreted as a consequence of UA dilator muscle contraction before twitch in these circumstances.

On the other hand, the improvement in the  $\dot{V}_{I_{\max, \lim}}$  when PNS was applied during inspiration cannot relate to the changes in driving pressure since one of the main characteristics of the flow-limitation regimen is the absence of a relationship between flow and driving pressure once  $\dot{V}_{I_{\max, \lim}}$  has been reached. We therefore attribute the differences in UA stability between in-

spiratory and expiratory twitches to the phasic UA traction forces. The very short difference in twitch timing makes it unlikely that the stabilizing effect of inspiration was due to differences in resistance upstream to the collapsing site, as suggested by the absence of difference in iso-flow pharyngeal resistance between inspiratory and expiratory twitches (Table 1). Furthermore, it must be emphasized that if any difference in UA area occurred, it would tend to be less during inspiration than during expiration (22), thus physiologically promoting UA collapse. This further supports the unique advantage of the PNS measurements that take into account the resulting effect of all the physiological conditions that contribute to determine UA stability during wakefulness.

Mouth aperture significantly modified inspiratory/expiratory difference in UA stability. We have previously found that during sleep mouth aperture increases the critical pressure by 3.7 cmH<sub>2</sub>O while exclusively breathing by the nose (16). According to the established relationships between upstream and downstream pressures and UA critical pressure, the decrease in  $\dot{V}_{I_{\max, \lim}}$  that occurred with mouth aperture without changes in limited-twitch P<sub>phar</sub> could be explained by an increase in upstream and/or downstream resistance. This is consistent with the rise in total respiratory resistance that accompanies mouth aperture during sleep (16), possibly as a consequence of the posterior movement of the mandible and the reduction in the oropharyngeal lumen with mouth opening (10). The major effect of mouth aperture on the inspiratory/expiratory difference in the percentage of twitches with a flow-limitation pattern supports the fact that mouth aperture alters the efficiency of UA dilating and stabilizing forces. The exact mechanisms that are involved in this detrimental effect cannot be determined from the present results, but it can be speculated that it could involve an alteration of the UA muscle operating length and/or the tracheal traction dilating effects as a consequence of the changes in UA shape and dimension.

The present results were obtained during wakefulness, and one could ask about the effects of sleep on the measured variables. Sleep can alter UA mechanical properties in many ways such as by decreasing tonic and phasic activities and decreasing lung volumes, with secondary changes in UA shape. Because all these factors are important determinants of the UA dynamic properties evaluated with PNS during both expiration and inspiration, it would be very difficult to anticipate whether sleep would alter the results of the two twitches differently. However, comparison of UA stability between inspiration and expiration provides valuable information of the effectiveness of the inspiratory dilating/stabilizing forces that can be compared between different subject/patient groups and between interventions aimed at modifying UA stability (i.e., modifying UA muscle activation pattern or contractile properties, changing soft tissues features). The ability to depict differences between apneic and nonapneic subjects with this technique during wakefulness would

demonstrate that disturbances in the mechanical effectiveness of inspiratory forces contribute to the occurrence of UA collapse during sleep.

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